A time-saving method for screening cDNA or genomic libraries

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The common procedure for isolating genes of interest is to screen cDNA or genomic libraries with nucleic acid probes by filter hybridization (1). However, this is a time consuming and labor-intensive process, especially when the complexity of the library is great and there is a large number of bacteriophage plaques to screen. Here we describe the use of the polymerase chain reaction (PCR) (2) to bypass some of the time-consuming steps of filter hybridization during the initial stage of library screening. This method enables one to reduce the complexity of a library by several orders of magnitude with only one round of plating and two rounds of PCR before any filter hybridization.

The cDNA or genomic library of interest is plated at a density of 50,000 pfu/150 mm plate as described (1). A replica is made by lifting a nitrocellulose filter from each plate. The filter is placed plaque site up in a sterile Petri dish and rinsed with 3 ml of lambda dilution buffer (0.1 M NaCl, 8 mM $MgSO_4$, 50 mM Tris-Cl pH 7.5, and 0.1% gelatin). An aliquot of 20 μl is removed from each filter rinse, boiled for 5 minutes, and then used as the template for PCR. Multiple plates can be processed simultaneously. The PCR products are analyzed by agarose gel electrophoresis. If a plate contains one or more phage plaques that have the target sequence, its corresponding lane in the gel will have a DNA band of the appropriate size. A nitrocellulose filter is then placed on this plate, and the filter as well as the agar is cut into several sections using a sterile scalpel blade. After marking each section, the filter sectors are lifted from the agar plate and rinsed separately with 1 ml of lambda dilution buffer. PCR is again performed on aliquots from each filter rinse and the products checked on an agarose gel. The recombinant bacteriophage of interest can be localized to one section. From this point on, the bacteriophage of interest can be isolated and purified either by traditional methods of plating and filter hybridization or by several more rounds of plating and PCR.

This method offers three advantages. (1) It reduces the complexity of the library by several orders of magnitude with just one round of plating and bypasses the exhaustive process of hybridization with large numbers of filters. To screen 1×10^6 plaques, for example, plating at 50,000 pfu/plate and cutting the positive plate into eight sections will localize the phage of interest to a pool of 6,250 bacteriophage plaques without any filter hybridization. (2) Since the search is for a DNA fragment and not simply a hybridization signal, it may reduce the time wasted plating and purifying false positives. (3) This method is particularly time-saving for isolating several genes/fragments at the same time, because multiple pairs of primers can be used in the same PCR reaction.

REFERENCES

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